CHAPTER - V

SPECTROPHOTOMETRIC DETERMINATION OF CAPECITABINE IN BULK AND DOSAGE FORMS

Capecitabine is an antineoplastic. It's officials status has been presented in Table.1.01 (p.2). The structural features, category, certain characteristics, therapeutic importance and commercially available formulations of CPTB are compiled in tables 5.01 (p. 240); 5.02(p, 257); and 5.03 (p. 259) respectively.

A very few physico- chemical methods appeared in the literature for the assay of CPTB in biological fluids and pharmaceutical formulations. Most of them are based on HPLC[294], LC[295-297], NIR[298,299] and UV, spectrophotometric methods. The analytically useful functional groups in CPTB have not been fully exploited for designing suitable visible spectrophotometric methods and so still offer a scope to develop more visible spectrophotometric methods with better sensitivity, selectivity, precision and accuracy. The author has made some attempts in this direction and succeeded in developing thirteen methods for the determination of CPTB using appropriate reagents such as M_3 (I_2/PMAP-SAc), M_4 (TA/PMAP-Cr (VI)), M_{14} (IO_4^-/MO (VI)/PMAP-SA), M_{22} (IO_4^-/MBTH). All these methods have been extended to pharmaceutical formulations as well.

A reported UV spectrophotometric method has been adopted for the determination of CPTB in pharmaceutical formulations (tablets) and used as reference method (Table 5.04,p.243) to compare the results obtained by the proposed methods.

Experimental:

1. Instruments used:

An ELICO, UV Visible digital spectrophotometer with 1cm matched quartz cells were used for the spectral and absorbance measurements. An Elico LI-120 digital pH meter was used for pH measurements.

Table 5.01

Structural features of selected drugs

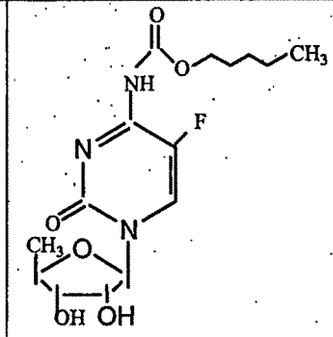
Drug Name / Abbreviation	Category	Chemical Name	Structure, Molecular formula and Molecular Weight	Analytically important moieties / functional groups
Capecitabine	Antineoplastic	5'-Deoxy-5-fluoro-n-[(pentyloxy)carbonyl)]cytidine;[1-(5-deoxy-β-D-ribofuranosyl)-5-fluoro-1,2-dihydro-2-oxo-4-pyrimidinyl]carbamic acid pentyl ester ; Pentyl1-(5-deoxy-β-D-ribofuranosyl)-5-fluoro-1,2-dihydro-2-oxo-4-pyrimidinecarbamate.		Vicinol diol,Pyrimidizone, Carbamic ester.

Table 5.02

Physico chemical characteristics and therapeutic importance of Capecitabine

Category	Characteristic	Therapeutic importance
Antineoplastic	Molecular formula $C_{15}H_{22}FN_3O_6$ Molecular weight - 395.35 State - A white crystalline powder Melting point -110-121^{0}c Solubility – Freely soluble in water, C_2H_5OH, Chloroform, methanol and 0.1N HCl, 0.1N NaOH Storage : Refrigerator UV_{max} : 280nm	The drug is currently used in the treatment of advanced breast cancer. The chemopotentiation of tesmilifene (Capecitabine) may be the result of pleitropic effects from ATP depletion including mdr1+ reversal and additive cytotoxicity with anticancer drugs.

Table 5.03

Commercially available formulations

Generic Name	Formulation	Strength of formulation	Other ingredients	
			Active	Inactive
Capecitabine	Tablet	500mg	Anhydrous lactose, Croscar mellose sodium, Hydroxypropyl methyl cellulose, Magnesium stearate, Purified water.	---
	Tablet	150mg	Hydroxy propyl methyl cellulose, Titanium dioxide, Talc, Red iron oxide, Synthetic yellow.	---

Table 5.04

Procedure for the assay of CPTB in formulations

Pharmaceutical formations	Reference Method
Tablets	An accurately weighed amount of formulation (Tablets powder) equivalent to 100mg was dissolved in a few ml of ethyl alcohol, evaporated to dryness and dissolved made up to 100 ml. 50 ml of this filtrate was further diluted to 100 ml with distilled water to obtained to a concentration of 500 µg/ml. It was further diluted step wise with distilled water to get the concentration of 25 µg/ml. Aliquots of CPTB solution 1.0-5.0ml, 25 µg/ml were taken into a series of 5 ml-calibrated tubes and made up to the mark with distilled water. The absorbance of each solution was measured at 280 nm against distilled water. The concentration of the drug was computed from its calibration graph.

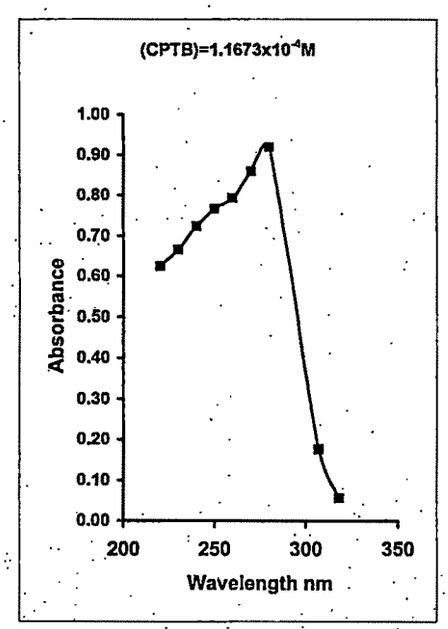


Fig. 5.01 Absorption spectrum of CPTB in Distilled Water (UV reference method)

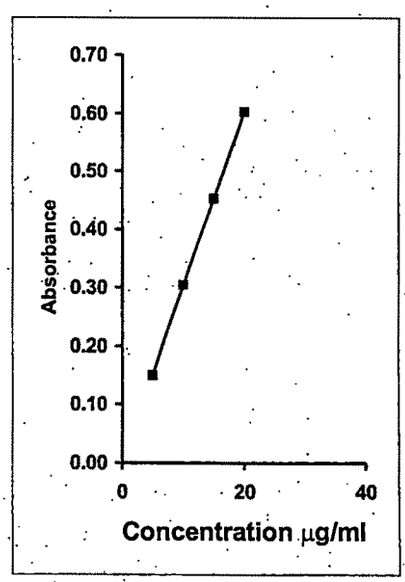


Fig. 5.02 Beer's law plot of CPTB in Distilled Water (UV reference method)

2. Preparation of standard drug solutions:

A 1 mg/ml solution was prepared by dissolving 100 mg of pure CPTB in 100 ml of distilled water and this stock solution was diluted step wise with distilled water to get the working standard solutions of concentration of 40 μg/ml (M_3, M_4), 200 μg/ml (M_{14}), 250 μg/ml (M_{22}).

3. Preparation of reagents:

All the chemicals and reagents used were of analytical grade and solutions were prepared in triply distilled water, isopropyl alcohol or chloroform.

Method M_3

Solutions of various reagents such as I_2 solution (E.Merk; 0.089%, $3.5x10^{-3}$ M), PMAP solution, (Loba; 2%, 5.807×10^{-2}M), SAc solution, (Sd-fine; 0.4%, $2.309x10^{-2}$M), Hydrochloric acid, (E.Merck, 1M) were prepared in the same way as described under RFB in Chapter – III (p. 151).

Method M_4

Solutions of various reagents such as TA solution (Loba 0.2%, $1.17x10^{-3}$M); PMAP solution, (Loba, 0.3%, $8.71x10^{-3}$M), Cr (VI) solution (BDH, 0.3%, $1.01x10^{-2}$M), Buffer solution pH = 3 were prepared in the same way as described under RFB in Chapter – III (p. 152).

Method M_{14}

$NaIO_4$ solution (BDH; 0.2%,$9.35X10^{-3}$M) : Prepared by dissolving 200mg of sodium meta per iodate in 100 ml of distilled water and standardized iodometrically.

Na_2MoO_4 solution (Qualigens; 1%, $4.13x10^{-2}$M) : Prepared by dissolving 1 gm sodium molybdate in 100 ml of distilled water.

PMAP solution (Wilson Labs; 0.3%, 8.71×10^{-3}M)	:	Prepared by dissolving 300 mg of p-N-methylaminophenol sulphate in 100 ml of distilled water.
SA solution (Sd-fine; 0.4%, 2.32×10^{-2}M)	:	Prepared by dissolving 400 mg of sulphanilamide in 5ml of 0.05M HCl followed by dilution to 100 ml with distilled water.
Buffer solution pH 3.0	:	Prepared by diluting a mixture of 250 ml of 0.2M potassium acid phthalate and 204 ml of 0.1 M HCl to 1000 ml with distilled water and the pH was adjusted to 3.

Method M_{22}

$NaIO_4$ solution (BDH; 0.2%, 9.35×10^{-3}M)	:	Prepared by dissolving 200 mg of sodium meta per iodate in 100 ml of distilled water and standardized iodometrically.
MBTH solution (Fluka; 0.2%, 8.56×10^{-3}M)	:	Prepared by dissolving 200 mg of MBTH in 100 ml of distilled water.
Acetic acid solution (Qualigens; 20%,v/v,3.49M)	:	Prepared by dissolving 20 ml of glacial acetic acid to 100 ml with distilled water.

4. Recommended procedures:

After systematic and detailed study of the various parameters involved, as described under results and discussions the following procedures M_3 (I_2/PMAP-SAc), M_4 (TA/PMAP-Cr (VI)), M_{14} (IO_4^-/MO (VI)/PMAP-SA), M_{22} (IO_4^-/MBTH) were recommended for the assay of CPTB in bulk samples and pharmaceutical formulations.

Method M_3

Aliquots of standard CPTB solution (0.5-3.0ml, 40 μg. ml^{-1}) were delivered into a series of centrifuge tubes. Then 2 ml of (1M) HCl and 2 ml of (3.5×10^{-3}M) I_2 were added successively. The volume was made up to 7ml with distilled water and kept aside for 15 min and centrifuged for 5 min. the precipitate was collected through filtration and subsequently washed with 2ml of distilled water. The filtrate and washings were collected in a 25 ml-

graduated tube. Then 3.0ml of PMAP ($5.807x10^{-2}M$) and 2.0 ml of SAc ($2.309x10^{-2}M$) solutions were added successively and the volume was made up to the mark with distilled water. The absorbance was measured after 25 min at 520 nm against distilled water. A blank experiment was also carried out omitting the drug. The decrease in absorbance and in turn the drug concentration was obtained by subtracting the absorbance of the test solution from the blank. The amount of CPTB was calculated from Beer's law plot (Fig. 5.07, p. 257).

Method M_4

Aliquots of standard drug solution (0.5-3.0ml 40 μg/ml) were delivered in to a series of centrifuge tubes and the volume in each tube was adjusted to 3.0ml with 0.01 N Hcl. Then 1.0ml of Tannic acid was added and centrifuged for 5 min. The precipitate was collected through filtration and subsequently washed with 2.0ml of distilled water. The filtrate and washings were collected in a 25ml-graduated test tube. Then 15ml of p^H 3.0 buffer and 1.5 ml of PMAP solution were successively added. After 2 min, 2.0 ml of Cr (VI) solution was added and the volume was made up to the mark with distilled water. The absorbance was measured after 5 min at 560nm against distilled water. A blank experiment was also carried out omitting the drug. The decrease in absorbance and in turn drug concentration was obtained by subtracting the absorbance of the test solution from the blank. The amount of drug was calculated from Beer's law plot (Fig. 5.08, p. 257).

Method M_{14}

Aliquots of standard drug solution (0.5-3ml, 200 μg ml) were delivered in to a series of 25 ml calibrated tube. To each tube 2ml of $NaIO_4$ reagent was added. Then heated on boiling water bath for 40 min. After cooling to room temperature 2ml of sodium molybdate, 10 ml of pH 3 buffer solutions were added and after 10 min. 1.5 ml of PMAP solution was added. After 2 min, 1ml of SA solution was added. The solutions in the calibrated tubes were made up to mark with distilled water. The absorbance was measured at 520 nm against

a reagent blank. The amount of drug was computed from its appropriate calibration graph (Fig. 5.09; p. 257).

Method M_{22}

Aliquots of standard CPTB solution (0.5-3ml, 250 $\mu g.ml^{-1}$), 1ml of $NaIO_4$ and AcOH were transferred into a series of 25ml-calibrated tubes. The volume was made up to the mark with distilled water and kept in boiling water bath for 40 min. The solutions were suddenly cooled. After that 1 ml of MBTH solution was added and kept aside for 15 min. Then diluted to 25 ml with distilled water. The absorbance was measured at 620nm against reagent blank. The amount of CPTB was computed from its calibration graph (Fig. 5.10, p. 257).

For pharmaceutical formulations:

An accurately weighed portion of tablet content equivalent to about 100 mg of CPTB was transferred into a 100ml volumetric flask. Added about 80 ml of warm distilled water and shaken well for about 20 min. The contents were diluted with distilled water up to the mark and mixed thoroughly. The solution was filtered and the filtrate was evaporated to dryness. The residue was used for the preparation of sample solution as under standard solution preparation. These solutions were analyzed as under procedures described for bulk solutions.

Results and Discussions:

i. Spectral Characteristics:

In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) of the colored species formed in the above methods, specified amounts of CPTB were taken and colors were developed separately by following the above procedures. The amounts of CPTB present in total volume of colored solutions were 50 $\mu g/ml$ (for method M_3), 4 $\mu g/ml$ (for method M_4), 10 $\mu g/ml$ (for method M_{14}), 8 $\mu g/ml$ (for method M_{22}).

The absorption spectra were scanned on a spectrophotometer in the wavelength region of 340 to 900 nm against similar reagent blank or distilled water. The reagent blank absorption spectrum of each method was also recorded against distilled water. The results were graphically represented in

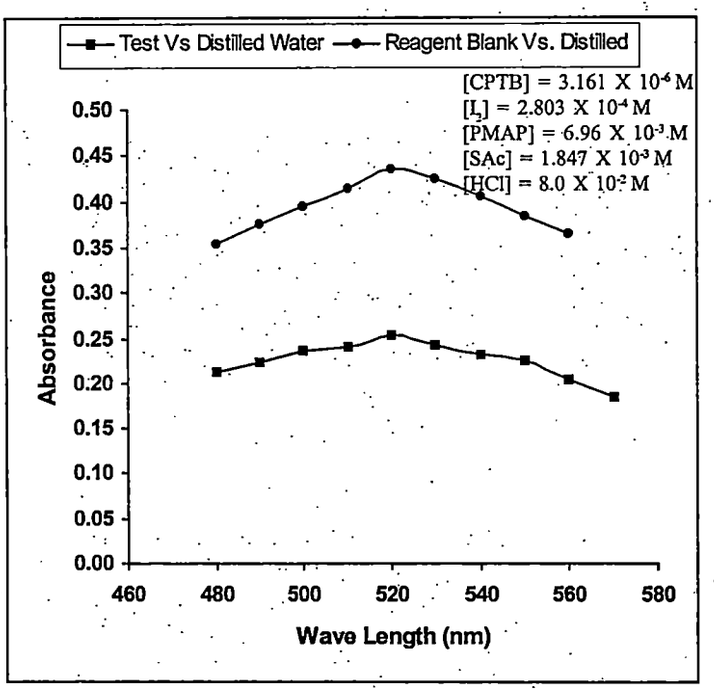


Fig. 5.03 Absorption spectrum of CPTB - I_2/PMAP - SAc (M_3)

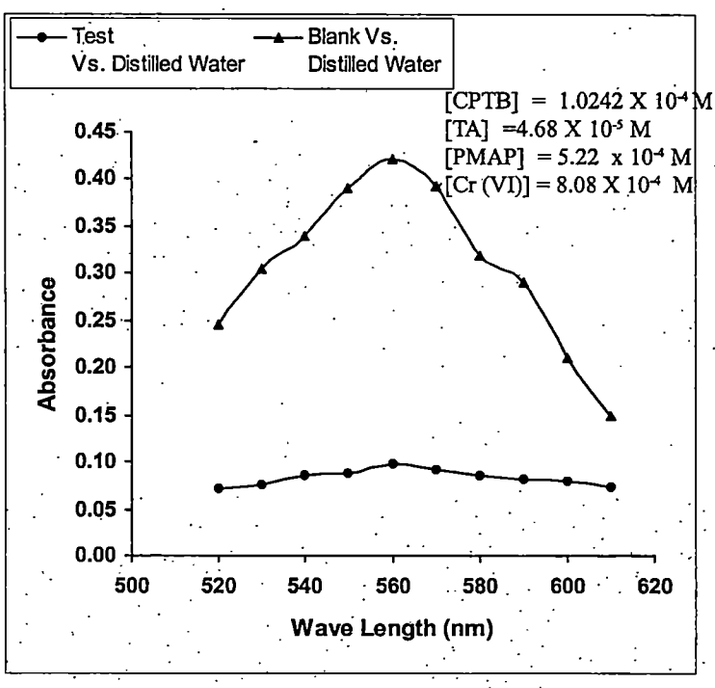


Fig. 5.04 Absorption spectrum of CPTB (TA/PMAP - Cr(VI)) (M_4)

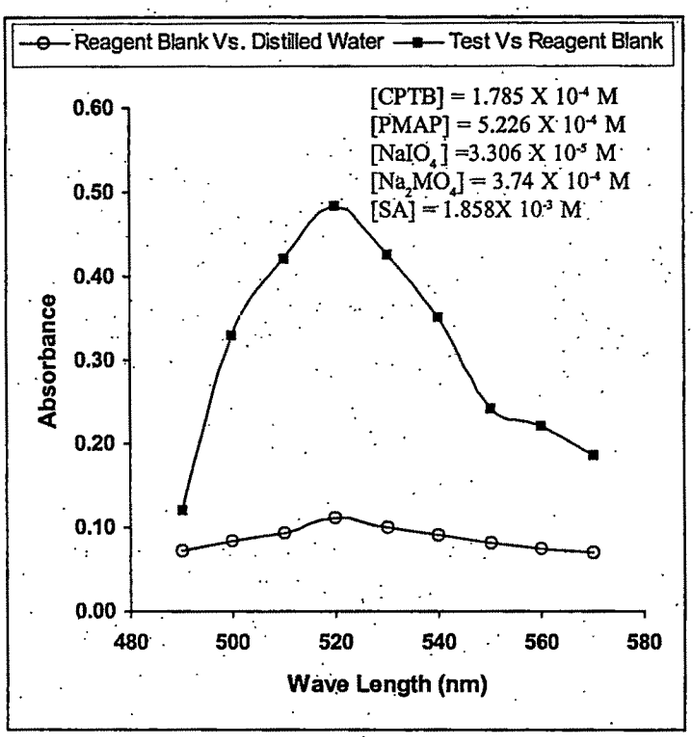


Fig. 5.05Absorption spectrum of CPTB - (IO_4^-/MoVI/PMAP-SA) (M_{14})

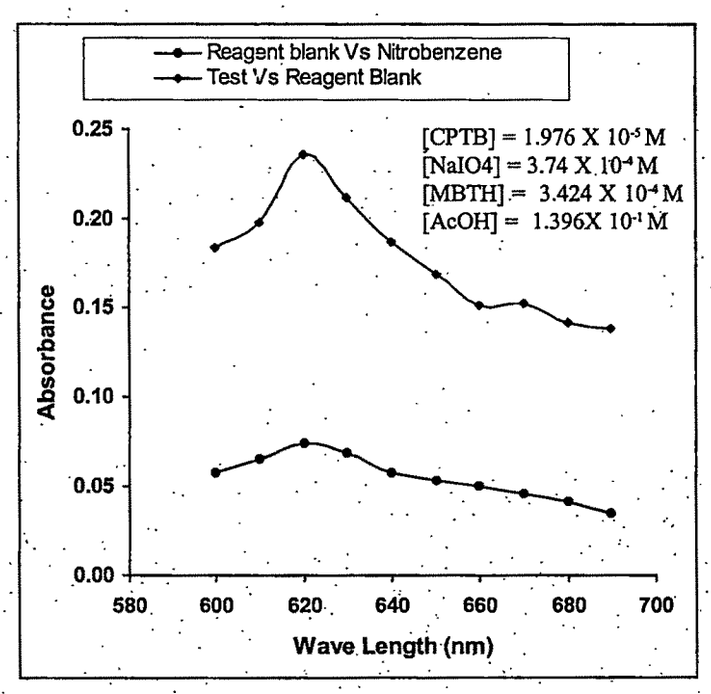


Fig. 5.06 Absorption spectrum of CPTB - IO4/MBTH (M_{22})

Fig. 5.03 to 5.06; p. 248-249. The absorption curves of the colored species in each method show characteristics absorption maxima (Table. 1.03, p. 14-18).

ii. Optimum conditions fixation in procedures:

The optimum conditions for the color development of methods (M_3, M_4, M_{14}, M_{22}) were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species. The following experiments were conducted for this purpose and the conditions so obtained were incorporated in recommended procedures.

Methods M_3, M_4

The optimum conditions established for methods (M_3, M_4) were found to be same as described in Chapter III (Table. 3.06, p. 176) (Table. 3.07, p. 177).

Method M_{14}

This method involves two steps, oxidation of drug by IO_4^- (first step) and estimation of the unconsumed IO_4^- with PMAP-SA reagent (second step).

In the first step, the volume of IO_4^- required for oxidation of drug, the time and temperature for heating the solution to complete oxidation were established through control experiments. In the second step, volume of sodium molybdate, pH, PMAP and intermittent time between additions and volume of SA, time and temperature for maximum color development, solvent for final dilution were found by varying one parameter at a time. The optimum conditions are incorporated in (Table 5.07,p.255)

Method M_{22}

In developing this method, a systematic study of the effects of various parameters were undertaken by varying one parameter at a time and keeping the others fixed, which is the standard practice in this type of work. The studies for this purpose include volume of $NaIO_4$, time and temperature required for oxidation

(prior to the addition of MBTH), volume of MBTH, effect of volume of AcOH, time and temperature for color development (after the addition of MBTH), order of addition, solvent for final dilution and stability of colored species formed were studied. The optimum conditions are incorporated in Table 5.08,p.256.

Optical Characteristics:

In order to test whether the colored species formed in above methods adhere to Beer's law, the absorbance's at appropriate wavelength of a set of solutions containing varying amounts of CPTB and specified of amounts of reagents were recorded against the corresponding reagent blanks. The Beer's law plots of these recorded graphically. Beer's law limits, molar absorptivity, Sandell's sensitivity and optimum photometric range for CPTB in each method were calculated. Least square regression analysis was carried out for getting the slope, intercept and the correlation coefficient values (Table 5.06a, p. 260).

Precision:

The precision of the proposed methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of CPTB in total solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods (Table 5.06a, p. 260).

Accuracy:

To determine the accuracy of each proposed method, different amounts of bulk samples of CPTB within the Beer's law limits were taken any analysed by the proposed method. The results (percent error) are recorded in (Table 5.10a-5.06a, p. 260).

Interference studies:

The effect of wide range of excipients and other active ingredients usually present in the formulations for the assay of CPTB in methods (M_3, M_4, M_{14}, M_{22}) under optimum conditions were investigated. The commonly used excipients and

other active ingredients usually present in formulations did not interfere even if they were present in amount than they usually exist.

Analysis of formulations:

Commercial formulations (tablets) containing CPTB were successfully analysed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically with F and t tests and found not to differ significantly. The results are summarized in (Table 5.11a, p. 261). Percent recoveries were determined by adding standard drug to prenalysed formulations. The results of the recovery experiments by the proposed methods are also listed in (Table 5.11a, p. 261).

Chemistry of the colored species:

The chemistry of the colored species formed in each one of the proposed methods for the assay of CPTB has been presented in chapter V.

Table 5.05

Optimum conditions established in method M_3 for CPTB

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	515-525	520	
Effect of volume ($3.5x10^{-3}$M) of iodine for the complete precipitation of CPTB	1.8-2.2 ml	2.0 ml	1.8ml of iodine was required for the complete precipitation of CPTB at upper Beer's law limits.
Volume of (1M) HCl	1.50 – 2.5 ml	2.0 ml	The development of color was slow with higher or lower acidity. 1.5-2.5ml was found to be best for attaining the high sensitivity.
Volume ($5.807x10^{-2}$M) of PMAP	3.0 – 4.0 ml	3.0 ml	3.0ml of p-N methylaminophenol sulphate was necessary for attaining the highest sensitivity.
Volume ($2.309x10^{-2}$M) of SAc	1.5-3.5 ml	2.0 ml	Lower volumes of sulphanilic acid delayed the attainment of maximum color intensity but gave longer period of stability, higher volumes speeded up color development but the duration of stability period was shortened.
Stability period of color after final dilution	25-48 min	25 min	The charge transfer complex possesses two components, PMBQMI (acceptor and oxidizing agent) and SAC (donor and reducing agent). So the stability of complex was less due to slow redox reaction.

Table 5.06

Optimum conditions established in method M_4 (for CPTB)

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	550-570	560	
Effect of acidity and volume of tannic acid (1.17×10^{-3}M) for precipitation	0.008-0.012N 0.8-1.2 ml	0.01 N HCl 1.0 ml	Decrease in the volume lead to low absorbance values. If the volume of tannic acid is increased abnormally, the precipitate formed partially dissolves in it by producing erratic results.
Effect of metol volume and color development of the filtrate	1.2-1.8 ml	1.5 ml	1.2 – 1.8 ml of (8.71×10^{-3}M) PMAP were found to be adequate for maximum color development.
Nature of oxidant on color development in combination with metol.	Cr VI 1.5 - 2.5 ml	Cr VI 2.0 ml	Oxidants such as Ce(IV), IO_4^-, $[Fe(CN)_6]^{3-}$, Fe (III). When used instead of Cr(VI) did not produce prominent colour. 1.5 – 2.5 ml of (1.01×10^{-2}M) Cr(VI) were necessary for maximum colour development.
Effect of time for maximum color development	2 - 5 min	5 min	2 min before and 5 min after addition of Cr(VI) gave maximum absorbance.
pH and volume of buffer on color development	13 - 17 ml pH 2.8-3.3	15 ml pH = 3	15ml of buffer of p^H=3 is necessary for getting constant and reproducible absorbance values.
Effect of order of addition of reagents	Buffer, Metol, Cr VI	Buffer, Metol, Cr VI	The absorbance is decreased if the order of addition is changed.
Nature of solvent for final dilution.	Water	Water	
Stability of colored species after final dilution.	5-40 min	5 min	

Table 5.07

Optimum conditions established in method M_{14} (for CPTB)

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	515-525	520	
Effect of volume of ($9.35x10^{-3}$M) $NaIO_4$ for oxidation	1.5-2.5ml	2.0ml	< 1.5 and > 2.5 ml of $NaIO_4$ solution produced low absorbance and high blank values respectively.
Time and temp required for oxidation	35-45min on boiling waterbath	40 min on boiling water bath	Heating time of 40 min. on boiling water bath was required to produce maximum color. Complete oxidation was not possible with in 40 min.
Effect of volume of pH 3 buffer solution	6-15 ml	10 ml	10ml of pH 3 buffer solution was necessary for maintaining the pH of the final colored solution.
Effect of volume of $4.13x10^{-2}$M sodium molybdate solutions.	1-3 ml	2 ml	< 1ml and > 3ml resulted in erratic absorsorbance values.
Effect of volume of $8.17x10^{-3}$M PMAP solutions.	1-2.5 ml	1.5 ml	<1 ml and>1.5ml PMAP solution produced low absorbance and high blank values respectively.
Effect of volume of $2.3x10^{-2}$M SA solutions.	0.75-1.25ml	1ml	A minimum amount of 1 ml of SA solution was necessary for CT complex formation with PMBQMI formed in insitu.....
Waiting time after addition of SA solution.	10-20 min at room temp	15 min	A minimum of 10 min. as waiting time was required for maximum color development.
Stability period after final dilution.	Immediately-60min	Immediately	After the stability period, the intensity of the colored species was found to decrease with time.

Table 5.08

Optimum conditions established in method M_{22} (for CPTB)

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	600-640	620	
Effect of volume of ($9.35x10^{-3}$M) $NaIO_4$ solution required	0.9-1.3 ml	1.0 ml	Addition of <0.9 ml and>1.3 ml results in low absorbance values.
Time and temp required for oxidation prior to the addition of MBTH	35-45 min on boiling water bath	40 min on boiling water bath	Heating time of 40 min. on boiling water bath was required to produce maximum color. At <30 min complete oxidation wasd not possible
Effect of volume of ($8.56x10^{-3}$M) MBTH required for color development	0.8-1.4 ml	1ml	Additionof <0.8 ml resulted in low absorbance especially at higher Beer's law limits. Increasing the volume beyond 1 ml has no effect.
Effect of volume of 3.49M acetic acid on color development.	0.8-1.2 ml	1ml	
Time and temp required for color development after the addition of MBTH	Kept aside for 10-20 min.	Kept aside for 15 min.	15 min of time was necessary to attain max color development.
Effect of order of addition of reagents on color development.	CPTB, $NaIO_4$, Acetic acid and MBTH	CPTB, $NaIO_4$, Acetic acid and MBTH	Order of addition has effect on the color development
Solvent for final dilution.	Water	Water	Distilled water is sufficient for final dilution, other water miscible solvents like methanol, ethanol, acetone etc.did not improve the color development to any extent.
Stability period after final dilution.	Immediately-45min	Immediate	

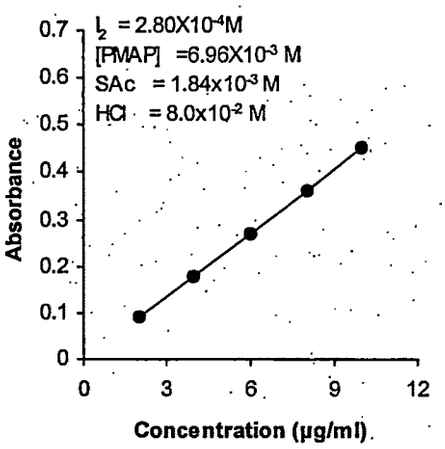


Fig.5.07 : Beer's Law plot of CPTB with I_2/PMAP-SAc system (M_3)

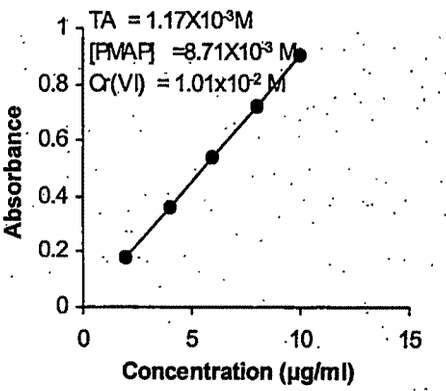


Fig.5.08 : Beer's Law plot of CPTB with TA/PMAP-Cr(VI) system (M_4)

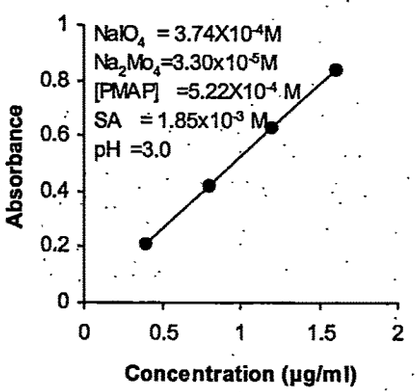


Fig.5.09: Beer's Law plot of CPTB with IO_4^-/MoVI/PMAP-SA system (M_{14})

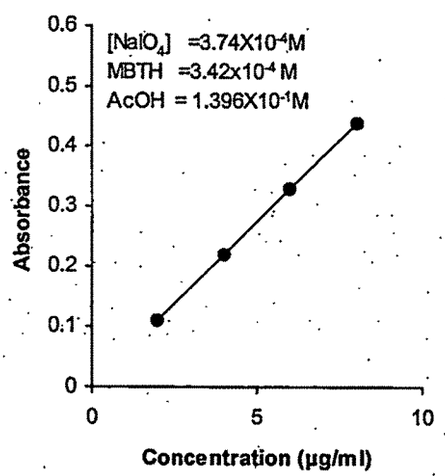


Fig.5.10 : Beer's Law plot of CPTB with IO_4^-/MBTH system (M_{22})

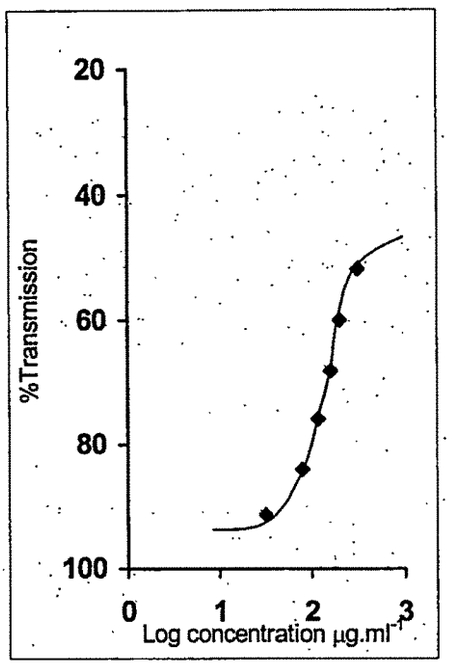


Fig 5.11 Ringbom plot of CPTB with I_2/PMAP-SAc (M_3)

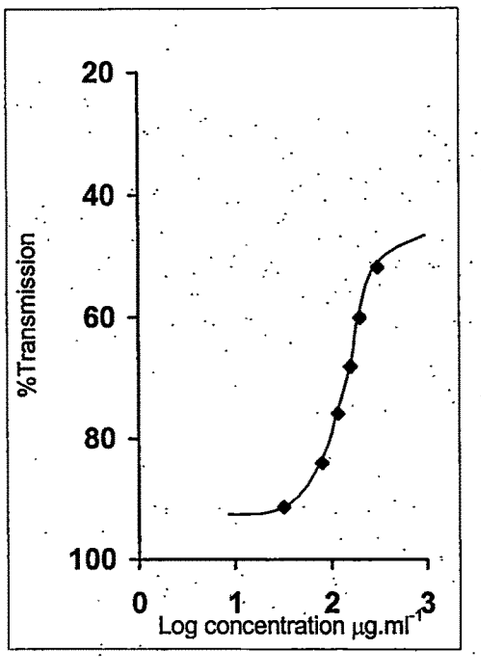


Fig 5.12 Ringbom plot of CPTB with TA/PMAP-Cr(VI) (M_4)

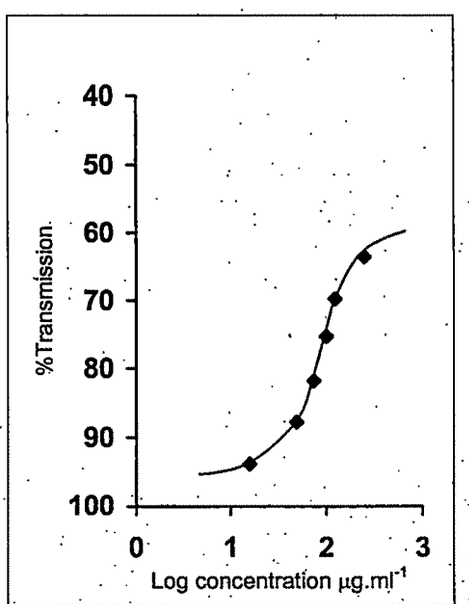


Fig 5.13 Ringbom plot of CPTB with IO_4^-/Mo(VI)/PMAP-SA (M_{14})

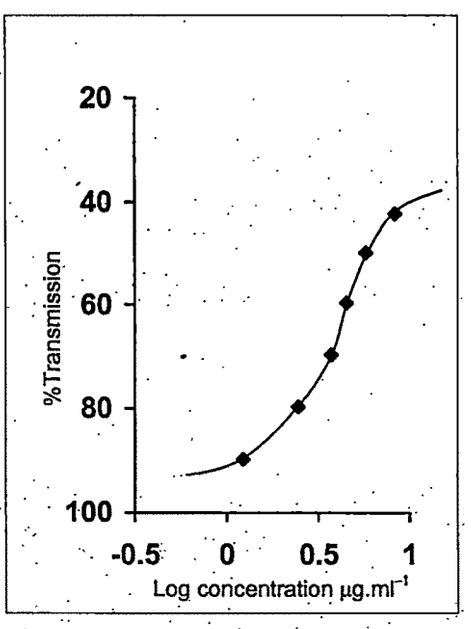


Fig 5.14 Ringbom plot of CPTB with IO_4^-/MBTH (M_{22})

Conclusions:

The proposed methods exploit the various functional groups in CPTB molecule. The decreasing order of sensitivity (ϵ_{max}) and λ_{max} among the proposed methods are (M_4>M_3>M_{14} >M_{22}) and (M_{22}>M_4>M_3 =M_{14}) respectively. The concomitants, which do not contain the functional groups chosen in the present investigation, do not interfere in the color development by proposed methods. Thus the proposed methods are simple, sensitive or selective with reasonable precision and accuracy and constitute better alternatives to the reported ones in the assay of CPTB in bulk form and pharmaceutical formulations.

Table 5.06a

Optical and regression characteristics, precision and accuracy of the proposed methods for CPTB

Parameter	M_3	M_4	M_{14}	M_{22}
λ_{max} (nm)	520	560	520	620
Beer's law limits (μg/ml)	2-10	1.5-10	0.5-1.75	2-8
Detection limit (μg/ml)	0.2667	0.2621	2.452	0.4274
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	2.226×10^4	2.601×10^4	2.072×10^4	8.732×10^3
Sandell's sensitivity (μg.cm^{-2}/0.001 absorbance unit)	0.6155	0.3012	1.464×10^{-1}	0.3661
Optimum photometric range (μg/ml)	40-125	126-250	31.62-112.2	25.12-120
Regression equation (Y=a+bc) slope (b)	0.0472	0.0747	0.4656	6.45×10^{-2}
Standard deviation on slope (S_b)	0.467×10^{-2}	0.9849×10^{-3}	2.8695×10^{-2}	1.385×10^{-3}
Intercept (a)	4.975×10^{-2}	1.89×10^{-2}	4.75×10^{-2}	6×10^{-3}
Standard deviation on intercept (S_a)	3.1004×10^{-2}	6.5335×10^{-3}	3.806×10^{-2}	9.190×10^{-3}
Standard error on estimation (S_e)	2.9564×10^{-2}	6.2294×10^{-3}	3.629×10^{-2}	8.7028×10^{-3}
Correlation coefficient (r)	0.9886	0.9590	0.9951	1.0000
Relative standard deviation (%)*	0.6165	0.3663	0.3077	0.5224
% Range of error (confidence limits)				
0.05 level	0.708	0.4214	0.3539	0.6006
0.01 level	1.111	0.6605	0.5549	0.9420
% error in Bulk samples**	0.10	0.102	0.125	0.196

* Average of six determinations considered

** Average of three determinations

Table 5.11a

Assay of CPTB in Pharmaceutical Formulations

Formulations*	Amount taken (mg)	Amount found by proposed Methods**				Reference method	Percentage recovery by proposed methods***			
		M_3	M_4	M_{14}	M_{22}		M_3	M_4	M_{14}	M_{22}
Tablet I	500	500.28±0.76 F=2.076 t=1.023	498.84±0.53 F=3.419 t=1.518	499.14±0.49 F=4.347 t=1.175	500.30±0.63 F=2.758 t=1.059	498.71±0.98	99.85±0.62	99.75±0.99	99.82±0.45	99.69±0.98
Tablet II	500	499.34±0.73 F=1.588 t=0.986	500.49±0.65 F=2.003 t=1.293	500.42±0.69 F=2.698 t=1.091	501.46±0.71 F=1.679 t=0.743	498.82±0.92	99.65±0.44	98.89±0.98	99.81±0.80	99.77±0.77
Tablet III	150	150.23±0.54 F=3.225 t=1.265	149.29±0.6 F=2.756 t=0.835	148.85±0.79 F=1.626 t=1.645	149.24±0.72 F=1.536 t=0.849	148.63±0.82	99.83±0.75	99.71±0.92	99.75±0.49	98.91±0.95
Tablet IV	150	149.53±0.86 F=1.538 t=1.861	150.35±0.7 F=1.852 t=0.706	148.95±0.77 F=2.017 t=1.897	148.58±0.68 F=2.419 t=0.725	149.97±0.93	99.65±0.62	99.85±0.99	99.72±0.45	99.79±0.98

* Tablets from four different pharmaceutical companies.

** Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.262

*** Recovery of 10mg added to the preanalysed pharmaceutical formulations (average of three determinations).

Capecitabine is chemically known as 5'-Deoxy-5-fluoro-n-[(pentyloxy) carbonyl)] cytidine. The analytically important functional groups in it are Vicinol diol in furan moiety (M_{14}, M_{22}) and hetero nitrogen's in pyrimidone moiety (M_3, M_4).

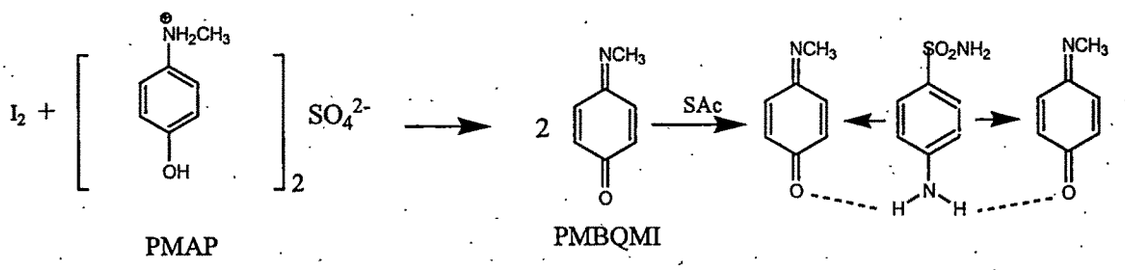
Method M_3

The method involves two steps. First step is the quantitative precipitation of CPTB with iodine. Second step is the formation of colored product between the unreacted iodine in the filtrate and the PMAP-SAc. The probable sequence of step reactions based on analogy are presented in the scheme 5.01

Step I

$$CPTB + I_2 \rightarrow \underset{\text{(Precipitate)}}{CPTB - I_2} + \underset{\text{(Unreacted)}}{I_2}$$

Step II



Scheme 5.01

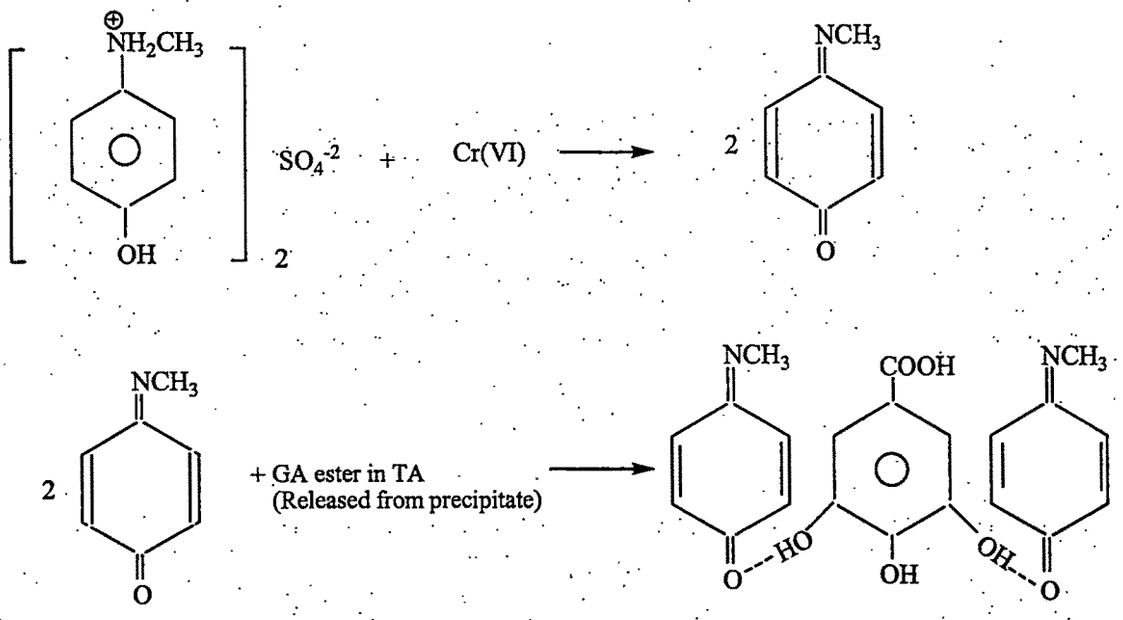
Method M_4

The method involves quantitative precipitation of CPTB with tannic acid. (Step I). The liberated tannic acid from the precipitate on treatment with acetone was determined with PMAP-Cr VI at pH 3.0. Tannic acid contains gallic acid units. It is probable that colored species originate through the involvement of PMBQMI (forms in situ from PMAP – Cr VI) and gallic acid unit in tannic acid in the formation of a charge transfer complex. The probable sequences of reaction based on analogy are presented in scheme5.02.

Step I

$$CPTB + TA \rightarrow \underset{\substack{\text{Adduct} \\ \text{(Precipitate)}}}{CPTB - TA} + \underset{\text{unreacted}}{T.A}$$

Step II

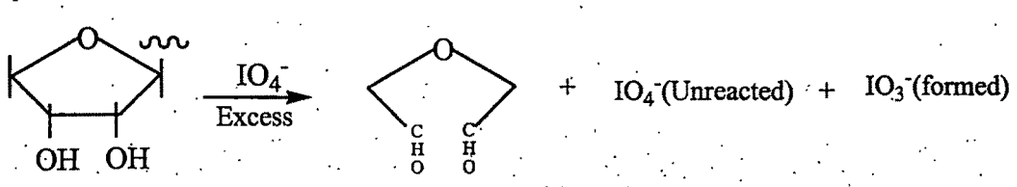


Scheme 5.02

Method M_{14}, M_{22}

The reactivity of CPTB towards IO_4^- appears to be mainly due to the presence of vicinal diol, in furan moiety. Oxidation of CPTB with periodate (M_{14}, M_{22}) was performed by heating on a boiling water bath till the completion of oxidation. The products of oxidation include dialdehyde and iodate (IO_3^- and reduced form of IO_4^-) besides unreacted IO_4^-.

Step I



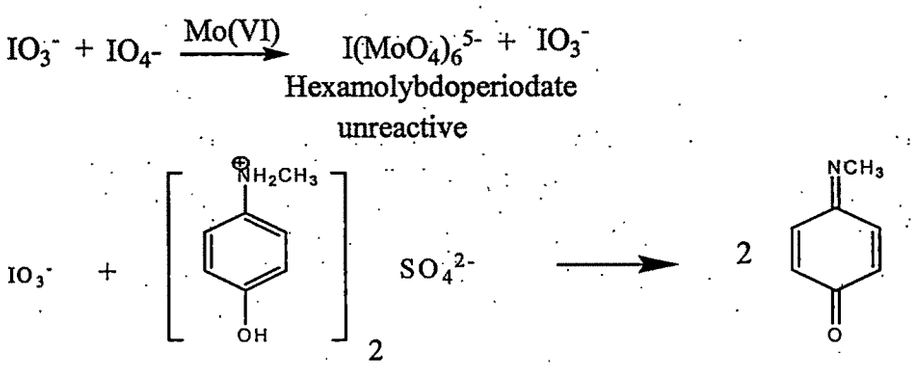
Vicinol diol portion of CPTB dialdehyde

Scheme 5.03

Method M_{14}

In method M_{14}, CPTB was determined by a method by involving oxidation with sodium meta periodate (Scheme 5.04,Step -I), masking the excess of periodate with sodium molybdate as hexa molybdo periodate and using PMAP-SA at pH 3 to determine IO_3^-. Based on analogy the color species formation in this step is shown in Scheme 5.04, Step-IIa.

Step-IIa

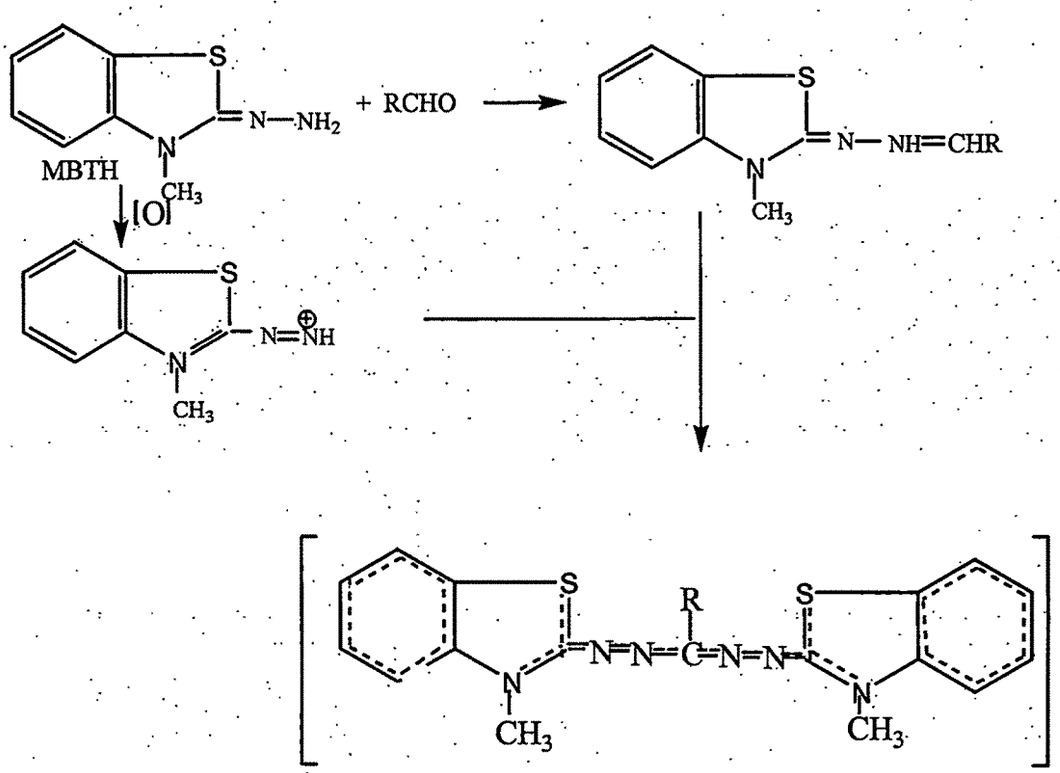


Scheme 5.04

Step-IIb

Method M_{22}

Method M_{22}, permits the determination of dialdehyde produced (Scheme 5.03,Step -I), directly in the reaction medium colorimetrically by oxidative coupling reaction with MBTH as already illustrated by Sawiki et al. The probable sequences of reaction based on analogy are presented in scheme 5.05, Step-IIb.



Scheme 5.05